High-throughput quantification of ten antiarrhythmic drugs in human plasma using UPLC-MS/MS

Bart van Domburg, Bart C.H. van der Nagel, Louise M. Andrews, Birgit C.P. Koch

PII: S1570-0232(18)31899-3

DOI: https://doi.org/10.1016/j.jchromb.2019.121793

Reference: CHROMB 121793

To appear in: *Journal of Chromatography B*

Received date: 27 December 2018

Revised date: 4 September 2019

Accepted date: 7 September 2019

Please cite this article as: B. van Domburg, B.C.H. van der Nagel, L.M. Andrews, et al., High-throughput quantification of ten antiarrhythmic drugs in human plasma using UPLC-MS/MS, *Journal of Chromatography B*(2018), https://doi.org/10.1016/j.jchromb.2019.121793

High-throughput quantification of ten antiarrhythmic drugs in human plasma using UPLC-MS/MS

Authors

Bart van Domburg[a], Bart C.H. van der Nagel[a], Louise M. Andrews[a], Birgit C.P. Koch[a]

Affiliations

[a] Department of Hospital Pharmacy, Erasmus MC, Dr. Molewaterplein 40, 3015 GD Rotterdam, Netherlands

Name and Address for Correspondence

Bart van Domburg, Research Technician
Department of Hospital Pharmacy
Erasmus MC, Postal box 2040 3000 CA Rotterdam, the Netherlands
Tel. +31 107032897, Fax +31 107032400
E-mail: b.vandomburg@erasmusmc.nl

Conflict of Interest and Source of Funding

The authors declare no conflict of interest.
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Highlights

- A fast and easy analytical LC-MS/MS method was developed for the simultaneous quantification of ten antiarrhythmic drugs in plasma.
- The compounds of interest are atenolol, bisoprolol, carvedilol, diltiazem, flecainide, lidocaine, metoprolol, norverapamil, propranolol, sotalol and verapamil.
- Sample pretreatment involves only protein precipitation and centrifugation.

ABSTRACT:

In this paper we present an FDA validated method to analyze ten antiarrhythmic drugs (atenolol, bisoprolol, carvedilol, diltiazem, flecainide, lidocaine, metoprolol, propranolol, sotalol and verapamil). A simple and fast sample preparation protocol with protein precipitation followed by ultra performance liquid chromatography (UPLC) for chromatographic separation and mass spectrometric detection applying electrospray ionization (ESI+) and selected reaction monitoring mode (MS/MS) was used. Only 50 µl plasma sample is needed for the simultaneous quantification of all compounds within a 5 min run-to-run analysis time. Sotalol-D_6, carvedilol-D_5 and verapamil-D_6 were used as internal standards.

The method was validated according to the FDA guidelines. Correlation coefficients were higher than 0.998 for all compounds. Intra- and interday accuracies were within 15 CV(%) for all analytes.
The method is currently successfully applied for routine analysis in our hospital.

Key Words: antiarrhythmic drugs; human plasma; LC-MS/MS; ultra performance liquid chromatography

1. Introduction

Antiarrhythmic drugs are used to suppress abnormal rhythms of the heartbeat (arrhythmias). Examples of arrhythmias are atrial fibrillation, atrial flutter, ventricular fibrillation and ventricular tachycardia. Some of the common symptoms of arrhythmias include heart palpitations and irregular heartbeats. Quality of life can be severely affected and burden of disease can be large. Most antiarrhythmic drugs have to be used for a long period. With these characteristics it is important that antiarrhythmic drugs are optimally used. Antiarrhythmic drugs are classified into five classes based on their mechanism of action (Singh Vaughan Williams Classification)[1,2,3].

Because of the different mechanisms of action of the drugs in each class, there is not one medicine to treat every kind of arrhythmia. Monitoring of antiarrhythmic drugs can be important because of the many different side effects, narrow therapeutic ranges and serious toxicity of these drugs [4,5,6]. Furthermore, the efficacy of beta-blockers and calcium-channel blockers has been proven, but the relationship between pharmacokinetics and pharmacodynamics (PK/PD) has not been fully investigated and needs more research.

A method for simultaneous determination of ten antiarrhythmic drugs and a metabolite using HPLC-MS/MS has been presented previously [7]. The method included protein precipitation of 100 µl sample and a 7.5 min gradient elution. The analytes of interest were amiodarone, atenolol, bisoprolol, carvedilol, diltiazem, metoprolol, mexiletine, propranolol, sotalol, verapamil and norverapamil. Besides most of the mentioned drugs, in clinical practice there is also a need of analyzing flecainide and lidocaine in our hospital. Our method was intended to be used for pharmacokinetic studies of carvedilol in plasma of children with heart failure as a result of dilated cardiomyopathy. Therefore, the method required relatively small minimum sample volumes. Additionally, a gradient elution of 7.5 minutes is not ideal in a large clinical laboratory. Therefore it was preferable that the total runtime should be shorter than the method presented by Li *et al* [7].

A multimethod for the analysis of ten antiarrhythmic drugs was developed to expand the range of antiarrhythmics analyzed in our lab and to improve our efficiency. We present a validated, rapid UPLC-MS/MS assay, developed for the simultaneous analysis of the following antiarrhythmic drugs: atenolol, bisoprolol, carvedilol, diltiazem, flecainide, lidocaine, metoprolol, propranolol, sotalol, verapamil and its active metabolite norverapamil.

2. Material and methods

2.1. *Chemicals and reagents*

Atenolol, carvedilol, diltiazem, and the hydrochlorides of propranolol, sotalol, sotalol-D_6 and verapamil-D_6 were purchased from Santa Cruz Biotechnology Inc (Heidelberg, Germany). Flecainide acetate, lidocaine HCl, metoprolol tartrate, norverapamil HCl and ammonium acetate were purchased from Sigma-Aldrich Chemie B.V. (Zwijndrecht, the Netherlands). Bisoprolol fumarate was purchased from Selleck Chemicals (Munich, Germany) and carvedilol-D_5 was purchased from @rtMolecule (Poitiers, France).
Methanol absolute LC-MS grade, acetonitrile HPLC Supra-gradient and formic acid 99% ULC/MS grade were purchased from Biosolve BV (Valkenswaard, the Netherlands).
Deionized water was prepared in our laboratory using a Millipore Milli-Q® Advantage A10® purification system (Merck Millipore, Amsterdam Zuidoost, the Netherlands).
Blank human plasma was obtained from the hematology laboratory of Erasmus MC (Rotterdam, the Netherlands).

2.2. Equipment

2.2.1 Instrumental

A Thermo TSQ Vantage UPLC-MS/MS system consisted of a Dionex Ultimate 3000 system coupled to a Thermo TSQ Vantage system triple quadrupole mass spectrometer equipped with a HESI-II probe. The UPLC system consisted of an Ultimate LPG-3400RS UPLC-pump, an Ultimate WPS-3000T RS autosampler and an Ultimate TCC-3000 RS column compartment (Thermo Scientific, Waltham, USA). Xcalibur 2.1 and Chromeleon 6.80 software were used for system control and data acquisition.
Samples were centrifuged with an Eppendorf 5415D microcentrifuge. A Liebisch evaporation manifold was used for the preparation of standards.

2.2.2. Chromatographic conditions

Chromatographic separation was achieved using a Waters Acquity BEH C_{18} column 2.1 × 50 mm, 1.7 µm particle size at a column oven temperature of 50 °C. A multistep gradient elution was applied at a flow rate of 0.5 ml/min using 2 mM ammonium acetate and 0.1% formic acid in water (mobile phase A) and 2 mM ammonium acetate and 0.1% formic acid in methanol (mobile phase B). The temperature of the autosampler was set at 15 °C. Gradient was optimized to obtain retention for the most polar analyte (sotalol), spreading of analytes throughout a short gradient, rinsing the column with 100% mobile phase B and conditioning the column for the next injection. The gradient program was employed as follows: 5-15% B (0-0.1 min), 15-50% B(0.1-0.15 min), 50-50% B (0.15-1.4 min), 50-100% B (1.4-1.5 min), 100-100% B (1.5-2.5 min), 100-5% B (2.5-2.7 min), 5-5% B (2.7-5.0 min).
Mass transitions for all analytes were determined by infusion of a stock solution in methanol. Parent mass was optimized and the three most abundant product ions were monitored. One MS/MS transition was selected for quantification of each analyte.

2.2.3. Mass spectrometry conditions

All analytes were detected using positive ESI in selected reaction monitoring (SRM)-mode. The capillary and vaporizer temperature were set at 250 °C and 400 °C, respectively. Sheath gas pressure and aux gas flow were set at 50 and 20 arbitrary units, respectively. Spray voltage was set at 3500 V and the collision gas pressure at 1.5 mTorr. Retention times, specific mass transitions, collision energies and S-lens voltages of all target compounds and internal standards are listed in Table 1.

2.3. Standards and quality control samples

2.3.1. Preparation of stock solutions

For each target analyte, two sets of 500 mg/l stock solutions were prepared in methanol . One set was used for preparation of calibration standards, the other for preparation of quality control samples. A working solution was made by combining appropriate amounts of each stock solution and evaporating the mixture to dryness at 40 °C under a stream of nitrogen. After evaporation, this solution was reconstituted in blank human plasma.

Stock solutions for carvedilol-D_5, sotalol-D_6 and verapamil-D_6 were prepared in methanol at concentrations of 4.0, 18 and 3.7 mg/l, respectively. The internal standard (IS) working solution was prepared by dilution using methanol/acetonitrile 1:1 (v/v). Final concentrations of carvedilol-D_5, sotalol-D_6 and verapamil-D_6 were 20, 90 and 18.6 µg/l respectively. All stock and working solutions were stored at -18 °C.

Sotalol-D_6 was used for quantification of atenolol, lidocaine and sotalol; carvedilol-D_5 was used for bisoprolol, carvedilol, diltiazem, flecainide, metoprolol and propranolol and verapamil-D_6 was used for quantification of norverapamil and verapamil. These three internal standards are chosen because their physical properties resemble those of the target compounds.

2.3.2. Preparation of calibration standards and quality control samples

The calibration curve was constructed by using eight calibration standards. The working solution served as the highest calibration level (std 8) and was diluted 2, 4, 10, 20, 50, 100 and 1000 times in blank human plasma. Quality control samples were prepared in blank human plasma at three levels. Concentrations of the highest and lowest calibration standards and the quality control levels are presented for each analyte in Table 2.

All calibration standards and quality control samples were filled out as 50 µl aliquots in 1.5 ml safe-lock Eppendorf® tubes and were immediately stored at -80 °C.

2.4. Sample Preparation

200 µl of IS working solution was added to 50 µl of plasma samples. After vortexing, the mixture was centrifuged at 16,000 *g* for 5 minutes. 100 µl of supernatant was transferred into an autosampler vial, diluted with 600 µl mobile phase A. 2 µl was injected into the UPLC-MS/MS system. Standards, QCs and

samples of flecainide and lidocaine were injected at 1 µl in order to reach the desired ULOQ without saturating the detector of the mass spectrometer.

2.5. Method validation

The method was fully validated for linearity, sensitivity, accuracy, precision, stability, recovery, matrix effects and carry-over according to the US FDA guidelines for bioanalytical validation [8].

2.5.1. Linearity

A calibration curve was constructed for each analyte using eight calibration standards in duplicate. The calibration curves were determined by peak area relative to the IS applying weighed (1/x) linear least square regression. The correlation coefficient (r) has to be at least 0.995 for all compounds.

2.5.2. Limits of Quantification

The lower limit of quantification (LLOQ) was defined as a preset subtherapeutic concentration that could be quantified with an acceptable accuracy and precision (≤20%). It was determined by analyzing six replicates of an LLOQ-standard. The upper limit of quantification (ULOQ) was defined as the highest calibration standard concentration with an acceptable precision and accuracy (≤15%). The ULOQ concentration should be above the therapeutic trough level of each analyte.

2.5.3. Accuracy and precision

Intraday accuracy and precision were examined by analyzing six replicates of each QC level. For inter-day precision, duplicates of each QC level were analyzed on six different days. Precision should not exceed 15% CV and accuracy should be within 15% of the nominal concentration.

2.5.4. Stability

Autosampler stability was examined by analyzing the extracts 24, 48 and 96 h after the first injection (t=0), using freshly prepared standards each time. Extracts were kept in the autosampler at 15 °C during the whole experiment. Measured concentrations of the stored extracts within 15% of the initial result are considered acceptable.

2.5.5. Recovery and matrix effects

Recovery (RE) and matrix effects (ME) were determined according to the method of Matuszewski [9].
Three sets of samples were prepared:
Set A: Standards in deionized water
Set B: Standards spiked after sample preparation in plasma of five different lots
Set C: Standards spiked before sample preparation in plasma of five different lots
Blanks, QC L and QC H were prepared and measured in duplicate for each set.
Matrix effects are calculated by comparing the quantitative results of set A and set B (B/A × 100%).
Recoveries are determined by comparing the quantitative results of set B and C (C/B × 100%). The efficiency of the total process is calculated by C/A x 100%.

2.5.6. Carry-over

Carry-over was investigated by analyzing a blank matrix sample directly after the highest calibrator. For each analyte, the calculated carry-over should be below 20% of its LLOQ.

3. Results and Discussion

This method was developed to simultaneously quantify ten antiarrhythmic drugs and was validated according to US FDA guidelines.

The samples were processed with a protein precipitation step using methanol, followed by LCMS analysis for quantification. The required sample volume of 50 µl human plasma and the short total run time of 5.0 min were a significant improvement to the method published by Li *et al* [7]. Additionally, our method has incorporated two compounds (flecainide and lidocaine) that are of great interest in our hospital. Chromatograms of the analytes at the LLOQ standard level are shown in Figure 1.

Injection volume of 2 µl was used for most compounds. An injection volume of 1 µl was used for flecainide and lidocaine in order to prevent saturation of the mass spectrometer at higher concentrations.

3.1. Linearity

Correlation coefficients and linear ranges of all analytes are listed in Table 3. Correlation coefficients were >0.998 and linear ranges adequately cover the therapeutic ranges of the analytes.

3.2. Limits of quantification

LLOQs are listed for each analyte in Table 3. All LLOQs were below the therapeutic trough levels and were quantifiable with an accuracy and precision within 20%. All ULOQs were above the therapeutic trough level. Precision at the ULOQ concentration level was within 15% CV for each compound and the accuracy was ≤15% of the nominal concentration (data not shown).

3.3. Accuracy and precision

Coefficients of variations of intra- and interday accuracies were within 15% at all QC concentrations of all analytes. At the low QC level, interday precision was 17.3, 15.2 and 16.3 CV(%) for metoprolol, carvedilol and norverapamil, respectively. Intraday precision of metoprolol was 15.8 CV(%) at QC L. During validation, the calculated LLOQs for carvedilol, metoprolol and norverapamil were higher than LLOQs based on earlier experiments, hence the prepared QCs at the lowest concentration level are close to the LLOQ resulting in higher CV(%). Intraday precision for all other analytes was ≤15 CV(%) (Table 3).

3.4. Stability

Autosampler stability of the extracts was at least 48 h for most analytes (data not shown). Exceptions were flecainide and lidocaine (at least 24 h) and carvedilol, metoprolol, norverapamil and sotalol (at least 96 h).

3.5. Recovery and matrix effects

Calculation of the matrix effects was based on duplicates of different lots of spiked pooled patient samples for each analyte. The results are presented in Table 4.
The matrix effect showed ion enhancement for carvedilol, diltiazem, flecainide, norverapamil and verapamil at QC L. The matrix effects (B/A) of the other analytes ranged between 89 and 112%. The recoveries (C/B) of verapamil, norverapamil, carvedilol and diltiazem were above the 115% threshold. Samples spiked with these compounds before sample preparation showed elevated signals compared to samples that were spiked after sample preparation. This result is unexpected because introducing the analytes before sample preparation normally results in no loss of that analyte. The reason behind this is not completely understood. However, it does not matter for the validity of the method, because the accuracy of all analytes was within 15% of the nominal value of the normal sample preparation.

3.6. Carry-over

The carry-over percentage was below 20% of the LLOQ for all compounds. The highest observed carry-over percentage was 12.9% for carvedilol. The calculated carry-over for the analyte with the highest response, flecainide, was 4.2% of its LLOQ.

3.7. Clinical practice

The validated method is now implemented for routine analysis on a weekly basis in our hospital. Patient samples were centrifuged and part of the plasma was sent to another laboratory (UMC Utrecht) for comparison. The remaining plasma was analyzed at our laboratory during routine analysis. The results are summarized in Table 5.
Comparison of results with another laboratory shows that our method produces reliable results. Our goal is to send patient samples to UMC Utrecht at least twice a year (based on availability) in order to continuously monitor the performance of our method.
After an extensive period of testing, we switched from self-made standards to commercially available standards ('MassTox® Antiarrhythmic Drugs', Chromsystems, Gräfelfing, Germany), as this is easier in practice. Also, commercially available QCs ('MassCheck® Antiarrhythmic Drugs', Chromsystems) are included in every week's analysis and these results are monitored for trends.

4. Conclusion

We have successfully implemented the described method for the analysis of ten antiarrhythmic drugs for therapeutic drug monitoring in our hospital. The method comprises low sample volume, simple protein precipitation sample pretreatment and fast LCMS analysis. The developed method is validated

according to the US FDA guidelines for bioanalytical methods. We believe this method is well suited for use in a clinical setting as well as for research concerning children with rhythmic problems.

References

[1] Singh BN, Vaughan Williams EM. A fourth class of anti-dysrhythmic action? Effects of verapamil on ouabain toxicity, on atrial and ventricular intracellular potentials, and on other features of cardiac function. *Cardiovasc. Res.* 1972;6: 109-119

[2] Harrison D. Antiarrhythmic drug classification: new Science and practical applications. *Am J Cardiol*. 1985; 56:185-187.

[3] Katritsis D, Camm J. Antiarrhythmic Drug Classifications and the clinician: a gambit in the land of chaos. *Clin. Cardiol.* 1994; 17:142-148.

[4] Campbell TJ, Williams KM. Therapeutic drug monitoring: antiarrhythmic drugs. *J. Clin. Pharmacol.* 2001; 52:21S-34S.

[5] Podrid PJ. Safety and toxicity of antiarrhythmic drug therapy: Benefit versus risk. *J of Cardiovasc. Pharmacol.* 1991; 17:S65-S73.

[6] Takada M, Goto T, et al. Appropriate dosing of antiarrhythmic drugs in Japan requires therapeutic drug monitoring. *J Clin Pharm Ther*. 2005; 30:5-12.

[7] Li S, Liu G, Jia J, et al. Simultaneous determination of ten antiarrhythic drugs and a metabolite in human plasma by liquid chromatography-tandem mass spectrometry. *J Chromatogr. B.* 2007; 847:174-181.

[8] US FDA. Guidance for Industry: Bioanalytical Method Validation, 2018.

[9] Matuszewski BK et al. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal. Chem.* 2003, 75:3019-3030.

Table 1. Retention times and specific MS settings for each target compound and IS

Compound	Retention Time (min)	Precursor ion (m/z)	Product ion (m/z)	Collision Energy (V)	S-Lens (V)
Sotalol-D_6	0.98	279.0	213.9	16	75
Sotalol	1.00	273.0	212.8	16	75
Atenolol	1.27	267.0	144.8	24	104
Lidocaine	1.54	195.0	57.6	34	90
Metoprolol	1.64	268.1	115.9	17	106
Bisoprolol	1.91	326.1	115.8	17	122
Propranolol	1.96	260.0	115.8	17	92
Flecainide	2.00	415.0	397.9	20	123
Carvedilol	2.09	407.1	99.8	28	139
Carvedilol-D_5	2.09	412.1	104.8	28	139
Diltiazem	2.18	415.0	177.8	24	114
Norverapamil	2.21	441.4	165.1	24	144
Verapamil-D_6	2.22	461.4	165.1	26	133
Verapamil	2.23	455.4	165.1	26	133

Table 2. Concentration of calibration standards and quality control samples

Compound	Std 1 (µg/l)	Std 8 (µg/l)	QC L (µg/l)	QC M (µg/l)	QC H (µg/l)
Sotalol	20	21240	83	664	5310
Atenolol	7.86	7860	33	262	2096
Lidocaine	50	53000	205	1641	13125
Metoprolol	1.11	1108	6.93	55.4	443.2
Bisoprolol	1.33	1334	10.4	83	667.2
Propranolol	2.55	2546	33	265	2122
Flecainide	100	10400	81	650	5200
Carvedilol	1.52	1517	11.9	95	758.4
Diltiazem	3.005	3005	19	150	1202
Norverapamil	1.41	1400	2.32	18.4	147.8
Verapamil	6.08	6084	32	254	2028

Table 3. Result summary of validation parameters.

Analyte	Correlation coefficient	LLOQ	ULOQ	Concentration Levels QCs	Intraday		Interday	
	r	(µg/l)	(µg/l)	(µg/l)	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
Atenolol	0.9984	20	7500	33	-0.6	8.9	-2.9	9.0
				262	-4.7	2.5	-2.5	3.3
				2096	-4.1	3.1	-3.1	3.0
Bisoprolol	0.9991	2	1300	10.4	5.7	4.1	0.3	9.1
				83.0	4.8	4.3	1.2	4.6
				667	0.8	5.6	-1.5	4.9
Carvedilol	0.9994	4	1500	11.9	0.3	9.0	7.1	15.2
				95.0	6.0	4.9	3.6	3.1
				758	5.3	5.2	2.9	5.1
Diltiazem	0.9993	10	3000	19.0	4.6	3.7	14.4	11.3
				150	5.5	3.7	11.4	3.8
				1202	1.6	5.4	9.2	5.0
Flecainide	0.9988	50	10000	100	-7.4	4.9	-3.9	5.9
				700	-7.4	10.1	-9.6	8.3
				5200	-10.2	1.0	-14.6	4.8
Lidocaine	0.9994	100	13130	200	2.0	2.9	4.1	7.8
				1600	2.9	10.8	2.2	8.4
				13100	-8.2	2.6	-10.6	4.6
Metoprolol	0.9997	10	1100	6.9	4.0	15.8	7.3	17.3
				55.0	-5.2	7.9	-9.4	4.8
				443	-12.8	3.0	-10.5	3.7
Norverapamil	0.9997	15	1400	2.3	-9.8	11.2	-8.1	16.3
				18.4	-2.2	4.3	-2.8	3.4
				148	-3.2	3.5	-1.7	3.5
Propranolol	0.9997	10	2500	33	7.5	4.0	9.7	6.9
				265	8.0	4.1	2.5	5.8
				2122	4.8	5.2	0.2	5.5
Sotalol	0.9997	50	20000	80	-0.9	3.9	-2.8	13.3
				660	-1.9	1.7	-1.4	3.0
				5310	-4.0	3.2	-3.6	2.9
Verapamil	0.9998	10	6000	32	8.3	3.4	9.9	8.1
				254	6.9	1.5	9.6	3.0
				2028	7.4	1.6	8.8	2.3

Table 4. Matrix effects and recoveries. A = standards in deionized water; B = standards spiked after sample preparation in plasma; C =standards spiked before sample preparation in plasma.

	Matrix effect (%) B/A		Recovery (%) C/B		Process Efficiency (%) C/A	
Compound	**QC L**	**QC H**	**QC L**	**QC H**	**QC L**	**QC H**
Atenolol	106	88	92	104	97	92
Bisoprolol	106	90	104	109	110	98
Carvedilol	148	88	119	119	176	105
Diltiazem	136	89	117	113	159	101
Flecainide	130	94	94	100	122	94
Lidocaine	112	96	91	102	103	98
Metoprolol	100	89	100	107	101	95
Norverapamil	142	78	159	124	227	97
Propranolol	89	84	102	100	91	84
Sotalol	101	98	99	96	100	94
Verapamil	162	83	164	124	267	103

Table 5. Examples of results of clinical samples measured in our laboratory. Samples were sent to an external lab (UMC Utrecht) for comparison.

Patient	**Age**	**Drug**	**Dose**	**Result (mg/L)**	**Result external lab (mg/L)**
1	58 y	Metoprolol	25 mg daily	114	115
2	3 m	Propanolol	3 x 7.5 mg per day	210	196
3	67 y	Verapamil	120 mg daily	773	755
		Norverapamil		216	224

Figure 1. Chromatograms of the ten antiarrhythmic drugs in human plasma at the LLOQ level. Retention times vary from 1.00 min (sotalol-D_6) to 2.29 min (verapamil).

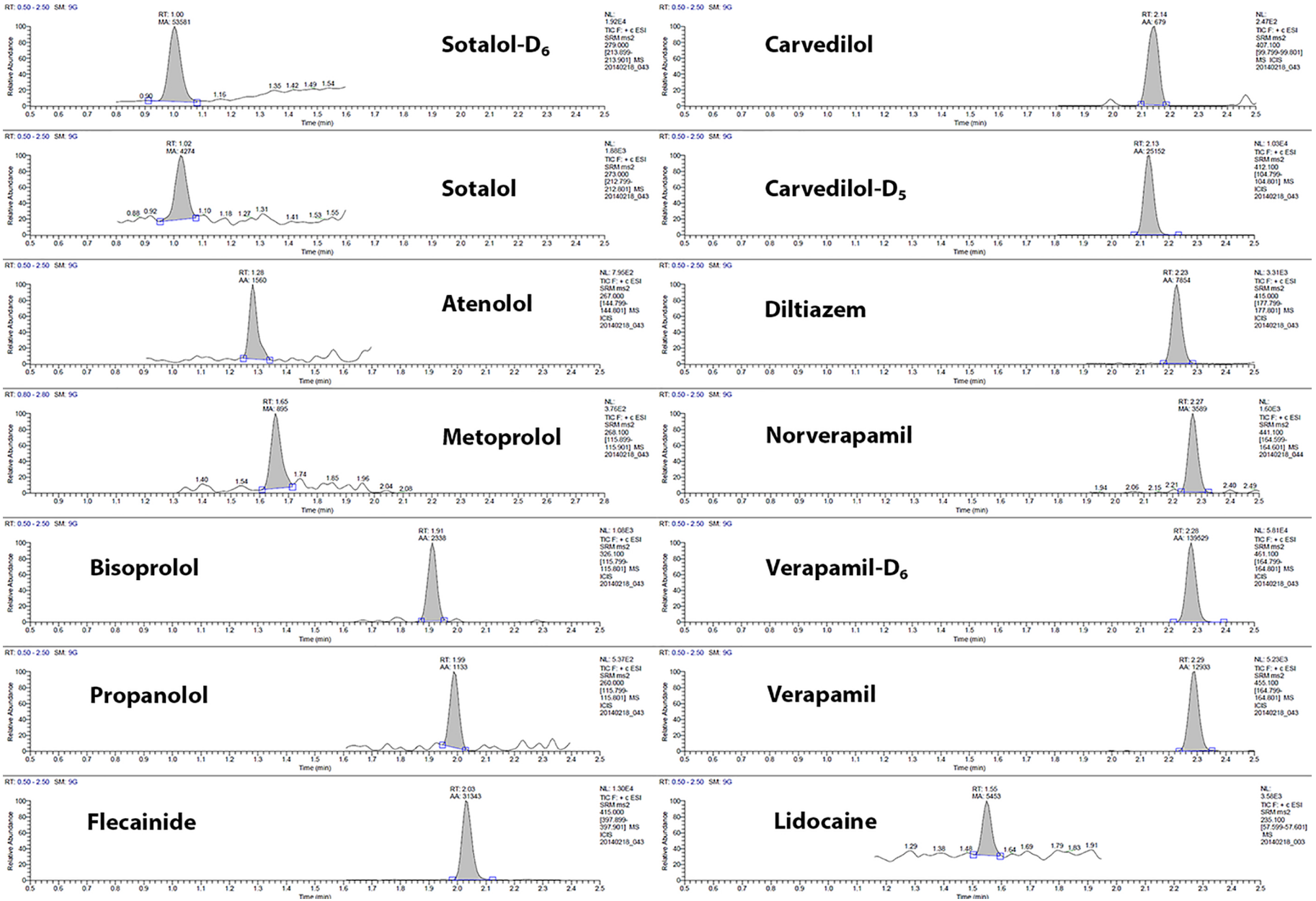
Sotalol-D6
Sotalol
Atenolol
Metoprolol
Bisoprolol
Propanolol
Flecainide
Carvedilol
Carvedilol-D5
Diltiazem
Norverapamil
Verapamil-D6
Verapamil
Lidocaine
Relative Abundance
Time (min)

Figure 1